AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Antioxidant and Antibacterial Activities of Essential oil and extract of *Zizyphora Hispanica* L. of M'sila

[1]Faiza Meratate, [2]Aissa Lalaoui, [3]Khellaf Rebbas, [1]Lotfi Benmakhbi, [3]Amina Bouhadda, [3]Bounab Souhila, [4]Salah Akkal, [1]El dhemi Ouali, [5]Hocine Laouer

[1]Department of chemistry, University of M'sila, Algeria
[2]University of Algiers, Algeria
[3]Department of Natural and Life Sciences, University of M'sila, Algeria
[4]Université frères Mentouri Constantine 1,UVRNAP, Faculté des Sciences Exactes, Département de Chimie, 25000, Constantine, Algérie
[5]Department of Natural and Life Sciences, University of setif1, Algeria

ARTICLE INFO

Article history:
Received 23 June 2015
Accepted 10 August 2015
Available online 25 August 2015

Keywords:
Lamiaceae, Zizyphora hispanica L., essential oil, Antioxidant and antibacterial activity

ABSTRACT

The increased interest in alternative natural substances is driving the research community to find new uses and applications of these substances. Zizyphora hispanica is a wide spread Mediterranean plant used in folk medicine to cure a variety of diseases and is widely distributed in Algeria; for that we study the antioxidant and the antibacterial activity of its extract and essential oils, The antioxidant activity determined by the DPPH test showed that The extracts reveal a relative high antioxidant capacity then the EO. The EO inhibits strongly the growth of Enterococcus faecalis ATCC 49452 and Acinetobacter banmanu ATCC 1966. The essence showed a strong antibacterial and antifungal activity.

To Cite This Article: Faiza Meratate, Aissa Lalaoui, Khellaf Rebbas, Lotfi Benmakhbi, Amina Bouhadda, Bounab Souhila, Salah Akkal, El dhemi Ouali, Hocine Laouer, Antioxidant and Antibacterial Activities of Essential oil and extract of Zizyphora Hispanica L. of M'sila. ***Adv. Environ. Biol., 9(14),*** *90-96, 2015*

INTRODUCTION

The genus *Zizyphora* L. belongs to the Lamiaceae family and it is represented in Algeria by three species of *Zizyphora* (*Z. capitata* L., *Z. hispanica* L. *and Z. tenuior* L.) according to Quezel and Santa (1963), and by four species (*Z. clinopodioides* Lam., *Z. capitata* L., *Z. persica* Bunge. and *Z. tenuior* L.) in Iran [13]. *Z. hispanica* have a local name: fleou or fleou el djebel and used as infusion and decoction and maceration or as powder for various purposes such as stomach and intestinal aches, heart disorders, migraine, cough and icterus as reported by [12]. In Turkish and Iranian folk medicine, *Ziziphora* species have been used as sedative, stomachic and carminative [16].

The pharmacological activities of *Ziziphora* have been investigated. Many studies have shown various activities for this genus such as antioxidant [11,18], antibacterial [7,19,16,1,18,10], anti-fungal [7]), insecticidal and ovicidal [9], vasodilating [21,22] activities. *Ziziphora* shows also an inhibitory effect on the gastric acid output in basal and vagal stimutated conditions [14], on performance, blood biochemical and immunity parameters of Laying Hens [15], ant it acts as yoghurt starter [8,20,14].

In recent years, antibacterial and antioxidant plant properties have gained special interest tanks to their richness of polyphenols which are considered as one of the important phytochemicals having antioxidant properties and having several industrial applications such as in the production of paints, paper and cosmetics, as tanning agents and in the food industry as additives and as natural antibacterial agents.

The aim of this study is to extract the principal active of *Z. hispanica and to* evaluate its antioxidant and its antimicrobial activities .

Chemicals reagents:

Dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and BHT (butylated hydroxytoluen), were purchase from Sigma-aldrich (St Louis, MO, USA). Methanol, n-butanol, petroleum ether,

Corresponding Author: Faiza Meratate, Department of chemistry, University of M'sila, Algeria.
E-mail: faizachimie@hotmail.fr

dichloromethane, from Fluka and prolab chemicals . Mueller Hinton agar from Oxoid Lab Ltd (Hamphire, England) and Sabouraud dextrose agar from Bacto-Difco Lab Co., Ltd (Detroit, MI, USA). All other chemicals were of the finest grade available.

Plant material:

Aerial parts of the *Z. hispanica* were collected from Boussaâda (M'sila) in June 2010. The taxonomic identification of plant species were confirmed by Dr. Rebass khellaf. The dried aerial parts were powdered and then used for extraction.

Microorganisms strains:

The following bacterial strains were used in this study: *Citrobacter freundii* ATCC 8090, *Bacillus cereus* ATCC 10876, *Staphylococcus aureus*, *Escherichia coli*, *klebsiella pneumoniae* ATCC 700603, *Salmonella typhimurium* ATCC 13311, *Enterococcus faecalis* ATCC 49452, *Listeria monocytogenes* ATCC 15313, *Proteus miralibis* ATCC 35659 and *Acinetobacter banmannii* ATCC 19606. Three fungi (*Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* were also tested.

Preparation of the extracts:

Z. hispanica powder was soaked, according Lebreto method (1967) modified by Boutard (1972), in 70% aqueous-methanol. The extract was filtered on filter paper then on sintered glass to obtain the first filtrate. This procedure was repeated twice on the residue using 80%, 90% aqueous –methanol respectively to obtain the last filtrate. The first and the last filtrates were combined then the methanol was removed under reduced pressure on a rotavapor below 45°C.

Crude extract (CrE) was subjected to fractionation using liquid-liquid extraction. CrE was successively extracted with different solvents of increasing polarity: petroleum ether for defatting, dichloromethane for aglycone flavonoids extraction and n-butanol for glycoside flavonoids extraction. The obtained organic layer of each partition was evaporated under reduced pressure on a rotavapor below 45°C to dryness and to afford petroleum ether, dichloromethane and n-butanolic fractions coded as PE, DE and BE, respectively.

Isolation of the essential oil:

250 g of dried aerial parts of *Z. hispanica* was submitted to water distillation for 3 h using a Clevenger-type apparatus. The distilled essential oils were stored at +4 °C for further use.

Antioxidant activity:

DPPH radical-scavenging activity:

The DPPH assay measures hydrogen atom (or one electron) donating activity and hence provides a measure of free-radical scavenging antioxidant activity. DPPH is a purple-colored stable free radical; it becomes reduced to the yellow-colored, diphenyl picryl-hydrazine [17]. According to Cuendet *et al.* [5] method with slight modification, 50µl of various dilutions of each extract or standards were mixed with 1250µl of a 0.004% methanol solution of DPPH. After an incubation period of 30 min in dark at room temperature, the absorbance of the samples was read at 517nm.

600µl of various dilutions of each essential oil were mixed with 600µl of a 0.004% methanol solution of DPPH. After an incubation period of 30 min in dark at room temperature, the absorbance of the samples was read at 517nm.

BHT wis used as positive control. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The ability to scavenge the DPPH radical was calculated by using the following equation:

Scavenging effect % =$((A_c - A_e)/A_c)\times 100$*:*

Where Ac: control absorbance and Ae: absorbance in the presence of extracts. IC_{50}values of the extract, concentration of extract necessary to decrease the initial concentration of DPPH by 50%, was calculated.

Antimicrobial activity:

Disc diffusion method:

Anti-bacterial activity of essential oil and methanolic extract was determined by the disc diffusion method [4] against many bacteria and fungi. Microorganisms were obtained from the culture collection of the Department of biology, University of Setif 1. Microorganisms were maintained on Muller-Hinton agar (MHA). Dimethylsulfoxide (DMSO) was used as negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18–24 h aerobically (Bacteria) and at 25°C for 7 days (fungi). After incubation, inhibition zone diameters were measured and documented.

RESULTS AND DISCUSSIONS

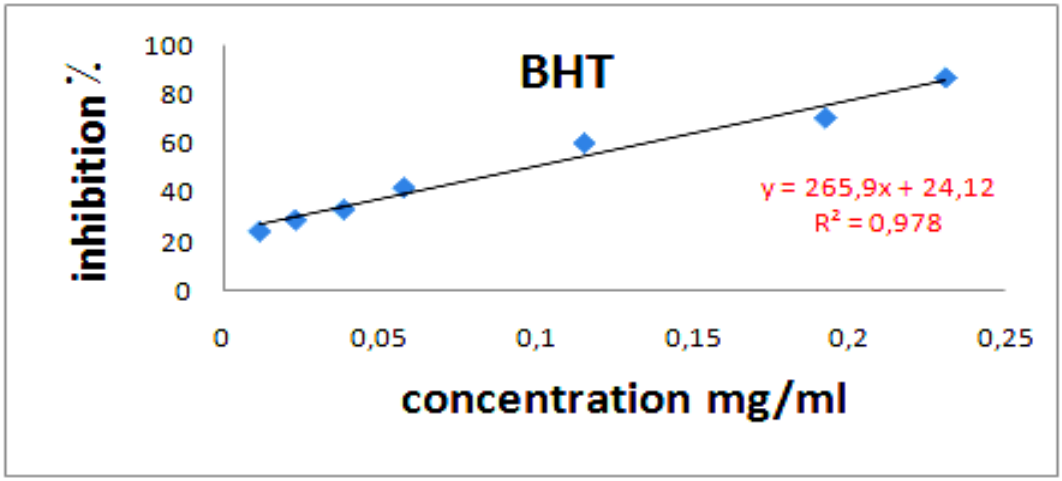
Antioxidant activity:

The DPPH radical is a free radical, which has been widely used as a tool to estimate free radical scavenging activity of antioxidants. Antioxidants, on interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thus neutralizing the free radical character [2].

Comparing IC_{50} of different extracts and HE with BHT standard. IC_{50} was:

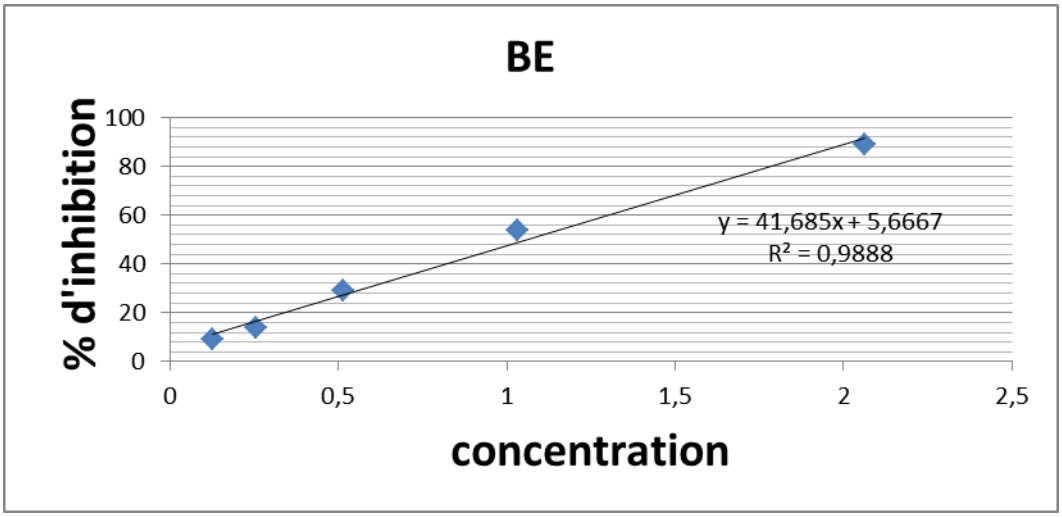
n-butanol extract (1.06 mg / ml)> petroleum ether extract (0.91 mg / ml)> extract dichloromethane (0.33mg / ml)> BHT (0.0059 mg / ml)> Eo (0.002 mg / ml).

However, the components responsible of the antioxidant activities of the oil were not identified and further work should be conducted to isolate these bioactive compounds.



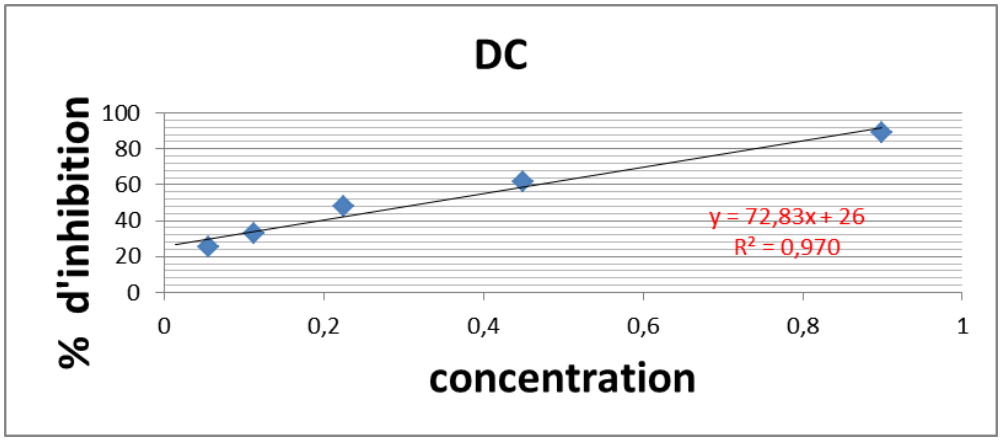
IC50 0,00594815

Fig. 1: Results of the antioxidant activity of BHT(Butylated hydroxytoluene).



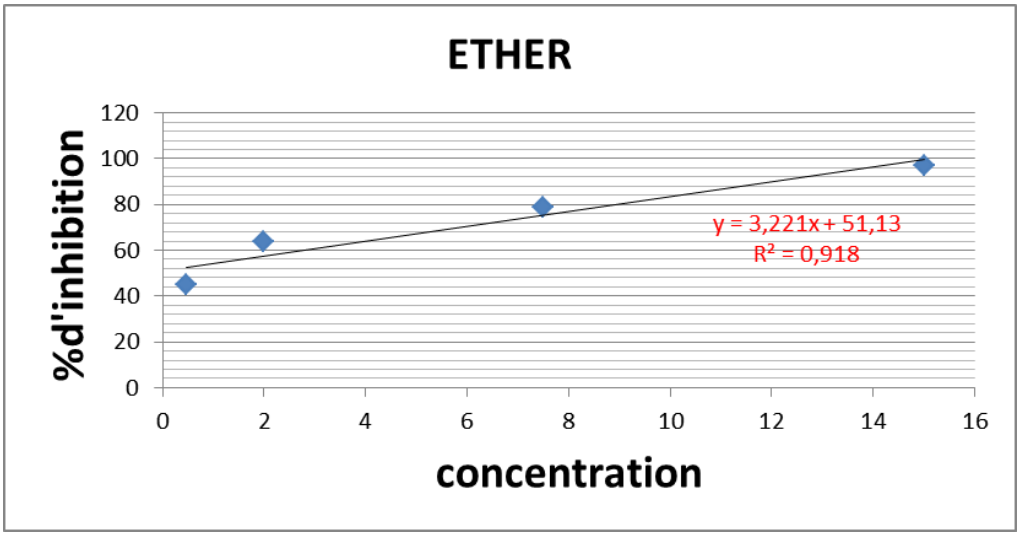
IC50 = 1,06353125 mg/ml

Fig. 2: Results of the antioxidant activity of the n-butanol extract.



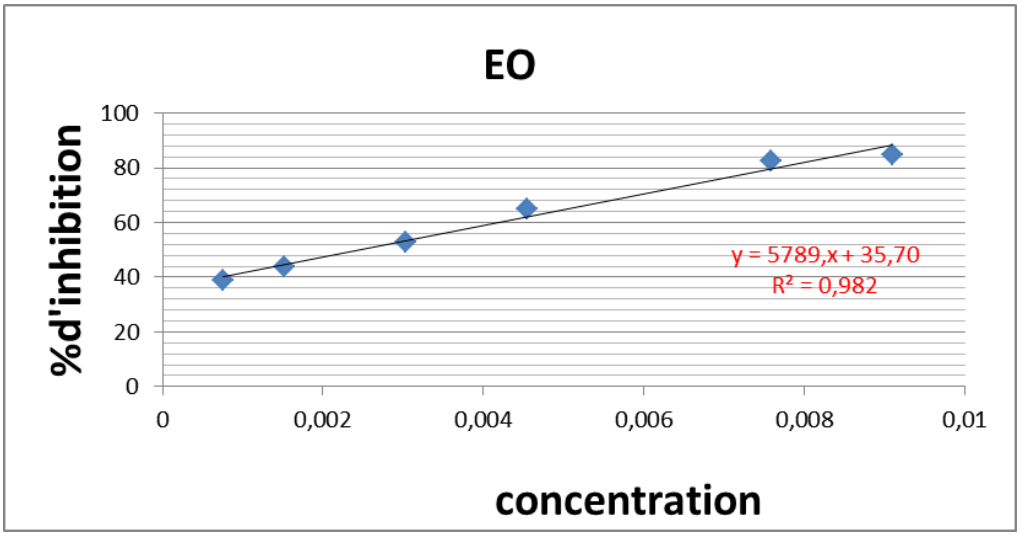
IC50 = 0,32952548 mg/ml

Fig. 3: Result of the antioxidant activity of dichloromethane extract.



IC50 = 0,91472849 mg/ml

Fig. 4: Results of the antioxidant activity of the petroleum ether extract.



IC50= 0,00236834 mg/ml

Fig .5: Results of the antioxidant activity of the essential oil

Antimicrobial activity:

The results regarding the antimicrobial activity of the essential oil ,dichlorométhane and n-butanolic extracts of *Z. hispanica* are indicated in Table 1. The results obtained from disc diffusion method indicated that Dichloromethane extract showed moderate antimicrobial activity against all microorganisms tested.

Candida albicans, *Entecococcus faecalis* ATCC 49452, *Acinetobacter baumannii* ATCC 1966 and *Staphylococcus aureus* were the most sensitive microorganisms with diameters of inhibition of 44, 44, 34 and 30 mm, respectively. On the other hand, *Citrobacter freundii* ATCC 8090 (26mm), *Escherichia coli* (26mm), *Aspergillus niger* (26mm), *Bacillus cereus* ATCC 10876 (24mm), *Aspergillus flavus* (24mm) and *Salmonella typhimurium* ATCC 13311 (18mm) were also sensitive to the oil. *Listeria monocytogenes* ATCC 15313 and *Klebsiella pneumonia* ATCC 70060 were the least sensitive microorganisms with 10 and 8 mm of diameters of inhibition respectively. The DMSO did not show antibacterial activity against the tested bacteria (negative control).

Surprisingly, in a previous study on essential oil of *Z. clinopodioides*, Shahla (2012) indicated that the plant seemed to be valuable sources for antibacterial drugs, especially against *Listeria monocytogenes.* When the fungal spore inhibition assay was applied to the oils , dichloromethane and n-butanolic extracts, observation during seven-day incubation period showed that *Aspergillus* spores were moderatly inhibited, while germination of *Candida albicans* were strongly inhibited by the tested samples.

Sonboli and their collaborators (2006) were tested the antibacterial activity of pulegone and 1,8-cineole against many bacteria and they concluded that the antibacterial activity of the oil may in part be associated with this two monoterpene. Pulegone has been shown to affect the cell membrane by dissipation of pH gradient and membrane potential of cells [3]. Javidnia and their collaborators (1996) indicate that the antimicrobial activity of the essential oil of *Ziziphora teniur* is mostly due to pulegone. It was shown that pulegone has pronounced activity against fungi and bacteria [6]. Thus, the antimicrobial activity of the oil may be associated principally with the relatively high pulegone (86.8%) content.

Based on these results, it is possible to conclude that aerial parts of *Z. hispanica* have a good antibacterial activity and antifungal against many microorganism. Thus, the essential oil of *Z. hispanica* have good antimicrobial effect like the essential oil of *Z. clinopodioides* on human pathogenic bacteria, indicating that the plant has potential use in phytotherapy.

Table 1: Antimicrobial activity of the extracts and EO of *Z. hispanica*.

	inhibition zone (mm)				
BE	DCE	E.Oil (10µ g/disk)	Gentamicin (10µ g/disk)	Bacterial strains	
12	18	26	13	*Citrobacter freundii* ATCC *8090*	1
10	12	24	18	*Bacillus cereus* ATCC 10876	2
6	20	44	30	*Enterococcus faecalis* ATCC 49452	3
12	8	18	15	*Salmonella typhimurium* ATCC 13311	4
8	12	8	/	*Klebsiella pneumoniae* ATCC *70060*	5
8	24	34	11	*Acinetobacter baumannii ATCC 1966*	6
10	8	18	/	*Proteus miralibis* ATCC 35659	7
10	10	10	11	*Listeria monocytogenes* ATCC 15313	8
10	12	26	21	*E. coli*	9
8	8	30	/	*S. aureus*	10
12	18	26	/	*Aspergillus niger*	11
12	12	24	/	*Aspergillus flavus*	12
8	20	44	/	*Candida albicans*	13

Conclusion:

Our results showed that, the extracts reveal a relative high antioxidant capacity then the essential oils (EO). The EO inhibit strongly the growth of *Enterococcus faecalis* ATCC 49452 and *Acinetobacter banmanu* ATCC 1966.The essential oils of *Zizyphora hispanica* have antioxidant activity higher than the control (BHT).While its extracts have an interesting antioxidant activity but remains lower than the standard BHT. In this study we also conducted a test in vivo antifungal EO of *Zizyphora hispanica* on tree fungi (*A. Niger*, *A.flavus* and *Candida albicans*), The results showed that essential oils have a very interesting antifungal activity especially with *Candida albicans* .

Zizyphora hispanica could be used as a source of natural polyphenols and antioxidants. According to the results we obtained, we can predict that essential oils are more antimicrobial while flavonoids are first class antioxidants.

Further investigation may be helpful in the use of the specific phenolic constituents of the essential oil as food preservatives, as aromatic foods imparts flavor and as natural antioxidants to reduce oxidative stress in human beings.

Determination of the natural antioxidant and antibacterial compounds of *Zizyphora hispanica* extracts will help to develop new drug candidates for antioxidant and antimicrobial therapy.

REFERENCES

[1] Aghajani, Z., F. Assadian, Sh. Masoudi, F. Chalabian, A. Esmaeilli, M. Tabatabaci-Anaraki and A. Rustaiyan, 2008. Chemistry of Natural compounds, 44(3): 387.
[2] Archana, B., N. Dasgupta, B. De, 2005. In vitro study of antioxidant activity of Syzygium cumini fruit. Food Chem, 90: 727–733.
[3] Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods- a review. Int. J. Food Micro, 94: 223-253.
[4] Collins, CH., PM. Lynes, JM. Grange,1995. Microbiological Methods. Butterwort-Heinemann Ltd. Britain, pp: 175-190.
[5] Cuendet, M., K. Hostettmann, W. Dyatmiko and O. Potterat, 1997. Iridoid glucosides with free radical scavenging propreties from Fagraea blumei. Helvitica Chimica Acta, 80: 1144-1152.
[6] Economou, D. and A. Nahrstedt, 1991. Chemical, Physiological, and Toxicological Aspects of the Essential Oil of Some Species of the Genus *Bystropogon*. Planta Med, 57(4): 347-351.
[7] Javidnia, K., M. Tabatabaiee and A. Shafiee, 1996. Composition and antimicrobial activity of essential oil of *Ziziphora teniur*, population Iran. DARU Journal of Pharmaceutical sciences, 6(1-2): 56-60.
[8] Khodaparast, H., M. Hossein, M. Sangatash, M.K.R.H. Najafi, M. Bagher and B.T. Shahram 2007. Effect of essential oil and extract of *Ziziphora clinopodioides* on yoghurt starter culture activity. World Applied sciences Journal, 2(3):194-197.
[9] Lolestani, F.A. and N. Shayesteh, 2009. Fumigant toxicity of *Ziziphora clinopodioides* (Boiss.) (Lamiaceae) against adults and eggs of *Callosobruchus maculates* (Fab.) (Coleoptera: Bruchdae). Journal of biological sciences, 9(1): 92-95.
[10] Mahboubi, M., S. Bokaee, H. Dehdashti and M.M. Feizabadi, 2012. Antimicrobial activity of *Mentha piperitae*, *Zhumeria majdae*, *Ziziphus teniur* oils on ESBLs producing isolates of *Klebsiella pneumonia*. Birarean biologist, 6(1): 5-9.
[11] Meral, G.E., S. Konyalioglu, B. Ozturk, 2002. Essential oil composition and antioxidant activity of endemic *Ziziphora taurica* subsp. Cleonioides. Fitoterapia, 73: 716–718.
[12] Meratate, F., 2012. Etude phytochimique et pouvoir biologique des métabolites secondaires de la plante *Zizyphora hispanica* L., mémoire de magistère, univ.de m'sila, Algérie.
[13] Mozaffarian,V.,1996. A dictionary of Iranian Plant Names. Farhang Moaser, Teheran, Iran.
[14] Niazmand, S., M. Derakhshan, M.E. Ahmadpour and K. Hosaeni, 2010. The inhibitory effect of *Ziziphora clinopodioides* Lam. On gastric acid output at Basal, vagotomized and vagal stimulated conditions in Rat. Iranian Journal of Basic Medical Sciences, 13(2): 36-39.
[15] Nobakht, A., N.H. Mansoub and M.A.M. Nezhady, 2012. Effect of *Melissa officinalis* L., *Tanacetum balsamita* L. and *Ziziphora clinopodioides* on performance, blood biochemical and immunity parameters of laying hens. Asian Journal of animal and Veterinary Advances, 7(1): 74-79.
[16] Ozturk, S. and S. Ercisli, 2007. Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides*. Food control, 18(5): 535-540.
[17] Prior, R.L, X. Wu and K. Schaich, 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 53: 4290-4302.
[18] Salehi, P., A. Sonboli, F. Eftekhar, S. Nejad-Ebrahimi and M. Yousefzadi, 2005. Essential oil composition, antibacterial and antioxidant activity of the oil and various extracts of *Ziziphora clinopodioides* subsp. *rigida* (Boiss.) Rech. F. from Iran. Biol. Pharm. Bull., 28: 1892-1896.
[19] Sanboli, A., M.H. Mirjalili, J. Hadian, S.N. Ebrahimi and M. Yousefzadi, 2006. antibacterial activity and composition of the essential oil of *Ziziphora clinopodioides* subsp. *bungeana* (Juz.) Rech. F. from Iran. Verlag der Zeitschrift für Naturforschung, Tübingen, pp : 677-680.
[20] Sarabi-Jumab, M. and R. Niazmand, 2009. Effect of essential oil of *Mentha piperita* and *Ziziphora clinopodioides* on *Lactobacillus acidophilus* activity as Bioyogurt starter culture. American-Eurasian J. agric and Environ. Sci, 6(2):129-131.
[21] Senejoux, F., C. Demougeot, P. Kerram, H.A. Aisa, Berthelot, F. Bévalot and C. Girard-Thernier, 2012. Bioassay-guided isolation of vasorelaxant compounds from *Ziziphora clinopodioides* Lam. (Lamiaceae). Fitoterapia, 83: 377-382.
[22] Senejoux, F., G. Corinne, P. Kerram, H.A. Aisa, A. Berthelot, F. Bévalot and C. Demougeot 2010. Mechanisms of vasorelaxation induced by *Ziziphora clinopodioides* Lam. (Lamiaceae) extract in rat thoracic aorta. Journal of ethnopharmacology, 132: 268-273.

[23] Shahla, S.N., 2012. Chemical composition and in vitro antibacterial activity of *Ziziphora clinopodioides* Lam. Essential oil against some pathogenic bacteria. African Journal of microbiology Research, 6(7): 1504-1508.